```
=> s liposome?(p)?butanol

          7629 LIPOSOME?
         57782 ?BUTANOL
L1         104 LIPOSOME?(P)?BUTANOL

=> s l1 and preliposom?

            10 PRELIPOSOM?
L2           2 L1 AND PRELIPOSOM?

=> d 1-2
```

1. 5,811,119, Sep. 22, 1998, Formulation and use of carotenoids in treatment of cancer; Kapil Mehta, et al., 424/450 [IMAGE AVAILABLE]

2. 4,950,432, Aug. 21, 1990, Polyene microlide pre-liposomal powders;
Reeta Mehta, et al., 264/4.6; 424/450, 502; 514/31, 37 [IMAGE AVAILABLE]

```
=> d 1-2 kwic
```

US PAT NO: 5,811,119 [IMAGE AVAILABLE] L2: 1 of 2

SUMMARY:

BSUM(7)

There . . . a retinoid for therapeutic purposes. For example, it is
often desirable to store the composition in the form of a **preliposomal** powder, but many prior formulations are not satisfactory
for such use, because they either contain an inadequate amount of retinoid,. . .

DETDESC:

DETD(7)

Prior to lyophilization, the carotenoid, lipids, and intercalation promoter agent can be dissolved in an organic solvent, such as t-**butanol**. Lyophilization to form a **preliposomal** powder can be performed using commercial apparatus which is known to persons skilled in
this field. After lyophilization, the powder can be reconstituted as,

liposome and (detergent? or surfactant?)

```
            5 PRELIPOSOME
        45035 DETERGENT?
        82178 SURFACTANT?
L1          2 PRELIPOSOME AND (DETERGENT? OR SURFACTANT?)

=> d 1-2
```

1. 5,741,513, Apr. 21, 1998, Alcoholic aqueous gel-like phospholipid composition, its use and topical preparations containing it; Miklos Ghyczy, et al., 424/450; 264/4.1, 4.3; 424/401; 514/944 [IMAGE AVAILABLE]

2. 5,711,965, Jan. 27, 1998, Alcoholic aqueous gel-type phospholipid composition, its use and topical preparation containing it; Miklos Ghyczy, et al., 424/450, 400, 401, 417, 420; 428/402.2 [IMAGE AVAILABLE]

e.g., **liposomes**, by adding a pharmaceutically acceptable carrier, such as sterile water, saline solution, or dextrose solution, with agitation, and optionally with. . .

US PAT NO: 4,950,432 [IMAGE AVAILABLE] L2: 2 of 2

ABSTRACT:
The present invention involves a process for producing fine powder suitable for the preparation of antifungal polyene microlide-containing **liposomes** upon suspension in an aqueous solution. This process comprises the following steps. Quantities of polyene macrolide and phospholipids are dissolved. . . mixture, for example by evaporation, to form a residue. The residue is then dissolved in a third solvent comprising tertiary **butanol** and methylene chloride to form a third solution. The third solvent is then removed from the third solution to form a remnant. The remnant is then dissolved in a solvent consisting essentially of tertiary **butanol** to form a fourth solution. The fourth solution is then filtered through a filter having orifices of between about 0.05 and 0.5 micrometers in diameter to produce a filtrate. The filtrate is lyophilized to remove the tertiary **butanol** and a fine powder remains. This fine powder may be used to form polyene macrolide-containing **liposomes** by simple incubation or suspension in an aqueous solution.

SUMMARY:

BSUM(24)

The present invention involves a process for producing fine powder suitable for the preparation of antifungal polyene microlide-containing **liposomes** upon suspension in an aqueous solution. This process comprises the following steps. Quantities of polyene macrolide and phospholipids are dissolved. . . mixture, for example by evaporation, to form a residue. The residue is then dissolved in a third solvent comprising tertiary **butanol** and methylene chloride to form a third solution. The third solvent is then extracted by evaporation from the third solution to form a remnant. The remnant is then dissolved in a solvent consisting essentially of tertiary **butanol** to form a fourth solution. The fourth solution is then filtered through a filter having orifices of between about 0.05 and 0.5 micrometers in diameter to produce a filtrate. The filtrate is lyophilized to remove the tertiary **butanol** and a fine powder remains. This fine powder may be used to form polyene macrolide-containing **liposomes** by simple incubation or

suspension in an aqueous solution.

DETDESC:

DETD(13)

A . . . frozen by immersion of a container in dry ice-acetone. The
frozen material was subjected to overnight lyophilization and a fine
preliposomal nystatin powder produced.

CLAIMS:

CLMS(1)

What is claimed is:

1. A process for producing a powder which forms **liposomes** comprising
an antifungal polyene macrolide upon suspension in an aqueous solution,
said process comprising the steps of:
(a) dissolving antifungal. . . in a quantity of a third organic solvent to form a third solution, wherein the third organic solvent
comprises tertiary **butanol** and methylene chloride;
(e) extracting the third organic solvent from the third solution to
leave a remnant;
(f) forming a fourth solution by dissolving the remnant in a solvent
consisting essentially of tertiary **butanol**;
(g) passing the fourth solution through a filter having orifices with
diameters of between about 0.05 um and about 0.5 um to produce a filtrate; and
(h) lyophilizing the filtrate to remove the solvent consisting essentially of tertiary **butanol**.

US PAT NO: 5,741,513 [IMAGE AVAILABLE] L1: 1 of 2

SUMMARY:

BSUM(11)

A recent method for the formation of liposomes consists in adding phospholipids in a cationic **detergent** to an organic solvent and transferring the lipid mixture to a finely divided or finely structured
surface such as molecular. . .

SUMMARY:

BSUM(16)

A so-called **preliposome** gel is obtained from a mixture of phospholipids, fatty acids and a hydrating agent according to EP-A-0 211
647. Liposomes. . .

US PAT NO: 5,763,585 [IMAGE AVAILABLE] L3: 21 of 62

DETDESC:

DETD(77)

A . . . thin lipid film. If desired, the film may be redissolved in a suitable solvent, such as tertiary butanol, and then **lyophilized** to form a more homogeneous lipid mixture which is in a more easily hydrated powderlike form. This film is covered. . .

DETDESC:

DETD(89)

Common . . . 9 and PLURONIC F-127.TM.(Wyandotte Chemicals Corp.). Preferred surfactants are nonionic polyoxyethylene and polyoxypropylene detergents compatible with IV injection such as, **TWEEN**-80.TM., PLURONIC F-68.TM., n-octyl-.beta.-D-glucopyranoside, and the like. In addition, phospholipids, such as those described for use in the production of **liposomes**, may also be used for micelle formation.

DETDESC:

DETD(93)

Preferably, . . . well-known sterilization techniques, or may be sterile filtered. The resulting aqueous solutions may be packaged for use as is, or **lyophilized**, the **lyophilized** preparation being combined with a sterile aqueous solution prior to administration. The compositions may contain pharmaceutically acceptable auxiliary substances as. . .

DETDESC:

DETD(100)

Kits . . . supplied for therapeutic or diagnostic uses. Thus, the subject composition of the present invention may be provided, usually in

a **lyophilized** form in a container. The complexes, which may be conjugated to a label or toxin, or unconjugated, are included in. . . .

DETDESC:

DETD(117)

Extraction . . . 20% acetic acid. The reaction mixture was centrifuged and the peptide pool supernatant was collected, frozen to -80.degree. C. and **lyophilized**. Reverse-phase high performance liquid chromatography (HPLC) was performed on a Waters (Millipore) 590 model using C-18 (0.21.times.15 cm) Vydac 218TP5215. . . of increasing acetonitrile from 10-60% in 0.1% trifluoroacetic acid (TFA). Acid-eluted HPLC peptide peak from post-Ni.sup.2+.NTA complexes was collected and **lyophilized**, and the identity was confirmed by integrated microsequencing using Porton PI2090 sequencer.

US PAT NO: 5,763,224 [IMAGE AVAILABLE] L3: 22 of 62

DETDESC:

DETD(23)

The novel fusions herein optionally are formulated into **liposomes** or other lipid membrane carriers. This is readily accomplished by mixing a solution of the GPI-linked fusion protein with a. . . fusions into the liposomal bilayer. Alternatively, the fusions are admixed with the aqueous solution used in the preparation of the **liposomes**. Alternatively, the fusions are formulated into conventional pharmacologically acceptable vehicles as described below for mDAF. Since the fusions bear hydrophobic substituent they can be formulated with pharmacologically acceptable detergents such as **Tween** 20 or polyethylene glycol (PEG), or with serum albumin. Such **liposome** fusions are especially useful in the treatment of infectious diseases and cancer therapy. For example, GPI-linked CD4 (CD4/DAF) can be. . . fusing the extracellular domain of CD4 to the GPI signal domain of DAF. The CD4/DAF may be linked to a **liposome** within which a toxic drug has been packaged, and then used to target the construct to HIV infected cells which express gp120 on their surfaces. Similar GPI fusions to ligands or antibodies can be used to target **liposome** containing toxic agent to cancer cells having receptors or antigens which specifically

bind to the ligands or antibodies.

DETDESC:

DETD(54)

DAF . . . ions as sodium, potassium, phosphate, chloride and the like. Generally, DAF is stored in phosphate buffered saline or may be **lyophilized** in the presence of an excipient including sugar alcohols, e.g. mannitol or sorbitol; monosaccharides, e.g., glucose, mannose, galactose or fructose;. . .

US PAT NO: 5,756,362 [IMAGE AVAILABLE] L3: 23 of 62

DETDESC:

DETD(26)

In . . . concentration sufficient to promote homogeneous flow of the test solution across the test device, to facilitate migration of the analyte analog-**liposome** conjugate without lysis of the **liposomes**. Suitable surfactants include Brij.TM. (polyoxyethylene ether), **Tween** 20.TM. (polyoxyethylenesorbitan monolaurate), Triton X-100.TM. (t-octylphenoxypolyethoxyethanol), sodium dodecylsulfate, n-octyl-.beta.-D-glucopyranoside, Span 20.TM., Nonindet P-40, Chapso.TM., Turgitol.TM. and sodium dioxycholate. The concentration of the surfactant(s) employed in a blocking solution will depend, in part, upon the **liposome** composition. In general, surfactants may be incorporated in a concentration of from about 0 to about 0.01 volume percent of. . . It is important that the concentration of surfactant applied to the absorbent material be controlled, as premature lysis of the **liposomes** may occur if the surfactant concentration is too high. **Tween** 20.TM. is a preferred surfactant for use in a blocking solution.

DETDESC:

D

US PAT NO: 5,820,880 [IMAGE AVAILABLE] L3: 13 of 62

SUMMARY:

BSUM(8)

Thus, in one aspect, the invention is directed to a pharmaceutical composition comprising at least one antigen encapsulated in **liposomes**, along with a stabilizing agent effective to prevent the disruption of the **liposomes** which would otherwise occur in the presence of alum. The stabilizer is a nonionic detergent. The structural characteristics of the nonionic detergent are such that it mimics the interactive properties of certain polyoxyethylene sorbitan esters, commercially known as "**Tweens.**" The esterified form of the **Tween** must contain less than 18 carbons in the acyl group and/or at least one .pi.-bond.

DRAWING DESC:

DRWD(3)

FIG. 2a shows the effect of **Tween** 80 concentration in **liposomes** on the release of glucose in the presence and absence of alum; FIG. 2b shows the effect of Span 80 concentration in **liposomes** on the release of glucose in the presence and absence of alum; FIG. 2c shows the effect of **Tween** 20 concentration in **liposomes** on the release of glucose in the presence and absence of alum; FIG. 2d shows the effect of **Tween** 40 concentration in **liposomes** on the release of glucose in the presence and absence of alum; FIG. 2e shows the effect of **Tween** 60 concentration in **liposomes** on the release of glucose in the presence and absence of alum; FIG. 2f shows the effect of **Tween** 65 concentration in **liposomes** on the release of glucose in the presence and absence of alum; FIG. 2g shows the effect of **Tween** 85 concentration in **liposomes** on the release of glucose in the presence and absence of alum.

DRAWING DESC:

DRWD(4)

FIG. 3 shows the time course of release of glucose from **liposomes** in

the presence of Alhydrogel.TM. for **liposomes** prepared with and without **Tween** 80.

DRAWING DESC:

DRWD(5)

FIG. 4a shows the effect of **Tween** 80 concentration on release of PSA from **liposomes** at two temperatures in the absence of alum; FIG. 4b shows the effect of **Tween** 80 concentration on release of PSA from **liposomes** at two temperatures in the presence of alum; FIG. 4c represents the difference between FIGS. 4b and 4a.

DETDESC:

DETD(5)

Especially . . . compositions can be stabilized by including, in the liposomal composition, a stabilizing agent which will effectively prevent the disruption of **liposomes** by contact with the adjuvant. The **liposomes** are formulated by using a stabilizing agent, typically a nonionic detergent corresponding to the properties of **Tween** 80. These properties are generated by detergent structures which contain polyoxyethylene side chains and which are free of saturated long-chain hydrophobic side chains. Thus, preferred nonionic detergent for inclusion in the liposomal preparations of the invention include **Tween** 20, **Tween** 40, **Tween** 80 and **Tween** 85.

DETDESC:

US PAT NO: 5,089,602 [IMAGE AVAILABLE] L3: 60 of 62

DETDESC:

DETD(14)

Apolipoproteins may be concentrated from solutions that contain them by various methods, e.g., membrane filtration or **lyophilization**. They may also be precipitated by adjusting the pH-value to 5 to 6 and/or the concentration of sodium chloride or. . .

DETDESC:

DETD(15)

Lipids . . . of the procedure, simultaneously with the suspension in an aqueous buffer, or at the very end of the procedure, with **lyophilized** product.

DETDESC:

DETD(17)

For . . . buffer substances like sodium phosphate, carbonate, or citrate in a physiological pH-range, may be acceptable. The solution may also be **lyophilized** and redissolved before use in a suitable solvent (water, buffer solution, salt solution, e.g., NaCl). It is also possible to. . .

DETDESC:

DETD(26)

0.5 . . . centrifugation (same conditions as above) was dialyzed against phosphate buffer (20 mM Na.sub.2 HPO.sub.4, pH 7.4, 150 mM NaCl) and **lyophilized**.

DETDESC:

DETD(30)

5 . . . (30 minutes, 0.degree. C.) and centrifuged (10 minutes, 0.degree. C., 10,000.times.g) and the supernatant was dialyzed against
phosphate buffer and **lyophilized** as described in example 1.

DETDESC:

DETD(33)

1 . . . then clarified by centrifugation or filtration; it was enriched in apolipoprotein A-I and could be further processed by dialysis
o

585,112 [IMAGE AVAILABLE] L3: 44 of 62

DRAWING DESC:

DRWD(90)

It . . . subsequent to being subjected to the methods of the present
invention. For example, the lipid may be hydrated and then **lyophilized**, processed through freeze and thaw cycles, or simply hydrated. In preferred embodiments, the lipid is hydrated and then **lyophilized**, or hydrated, then processed through freeze and thaw cycles and then **lyophilized**, prior to the formation of gaseous precursor-filled liposomes.

DRAWING DESC:

DRWD(147)

In . . . as an aid to the gaseous precursor instillation process as
well as to maintain the stability of the gaseous precursor-filled **liposomes**, for example, emulsifiers may be added to the lipid. Examples of emulsifiers include, but are not limited to, glycerol, cetyl.
. . glycol, propylene glycol, ethyl alcohol, sodium lauryl sulfate,
Laureth 23, polysorbates (all units), all saturated and unsaturated fatty
acids, triethanolamine, **Tween** 20, **tween** 40, **Tween** 60, **tween** 80, Polysorbate 20, Polysorbate 40, Polysorbate 60, and Polysorbate 80.

US PAT NO: 5,653,996 [IMAGE AVAILABLE] L3: 36 of 62

SUMMARY:

BSUM(8)

Several . . . Bioeng., 9:467-508 (1980). Among the more common of these are 1) sonication of a solution containing lipids sometimes followed by evaporation/**lyophilization** and rehydration (see, e.g. Stryer, Biochemistry, pp. 290-291 (Freeman & Co., New York, 1988), and Ohsawa et al., Chem. Pharm.. . . Jun. 1984, U.S. Pat. No. 4,895,719 issued 23 Jan. 1990, and U.S. Pat. No. 4,946,787 issued 7 Aug. 1990), 4) **lyophilization** or evaporation and rehydration (see e.g. U.S. Pat. No. 4,897,355 issued 30 Jan. 1990, EP 267,050 published 5 Nov. 1988,. . .

SUMMARY:

BSUM(24)

The . . . further processed, such as by formulating the suspension for use, or introducing the liposomes into vials or other containers and **lyophilized** or otherwise readied for storage. For extended storage, it is currently preferred that the product be **lyophilized** and kept below the temperature at which the frozen molecules are immobile and below any phase transitions.

DETDESC:

DETD(52)

The . . . be further processed, such as by formulating the suspension as described infra, or filled into vials or other containers and **lyophilized** for storage. The product bottles or other containers may be provided sterile. For extended storage, it is currently preferred that the **lyophilized** product be kept below the temperature at which the frozen molecules are immobile and below any phase transitions.

DETDESC:

DETD(53)

Lyophilization cycles typically involve a freezing cycle (at about -550.degree. C.) for several hours, followed by one or more drying cycles,. . .

DETDESC:

DETD(56)

For therapeutic use, the **liposomes** are placed into pharmaceutically acceptable, sterile, isotonic formulations together with required cofactors, and optionally are administered by standard means well. . . formulation is preferably liquid, and is ordinarily a physiologic salt solution containing non-phosphate buffer at pH 6.8-7.6, or may be **lyophilized** powder. **Liposomes** may be formulated with pharmacologically acceptable detergents such as **Tween** 20 or polyethylene glycol (PEG), or with serum albumin.

DETDESC:

DETD(61)

It . . . tubing is also used. Alternative routes include tablets and the like, commercially available nebulizers for liquid formulations, and inhalation of **lyophilized** or aerosolized liposomes. Liquid formulations may be utilized after reconstitution from powder formulations.

DETDESC:

DETD(98)

G. **Lyophilization:**

DETDESC:

DETD(100)
2. The **lyophilization** cycle was:

DETDESC:

DETD(110)

Several analyses of the liposome suspension after **lyophilization** were made. The residual moisture was determined in the dry **lyophilized** cake. Next, the product was resuspended in either water or saline. A particle size analysis was then done (described below),. . .

DETDESC:

DETD(112)
The residual moisture averaged in the 2.5-3% range for the **lyophilized** SP-C liposome cake prepared by the standard **lyophilization** cycle just described. An alternate cycle (Primary Drying at -24.degree. C., 320 .mu.m Hg for 14 hrs), performed with a. . . range. Liposome products produced with both cycles performed well in both in vivo and in vitro surfactant function tests. The **lyophilization** cycle chosen above is considered more conservative with a lower product temperature of -33.degree. C. during initial primary drying, but. . .

DETDESC:

DETD(114)
Reconstitution of the **lyophilized** SP-C liposome cake using SWFI water provided a stable liposome suspension. The particle size of the liposomes was determined in. . .

DETDESC:

DETD(115)

The . . . weight average results show a distribution in the 20-50 .mu.m range, depending on whether or not the suspension has been **lyophilized**. The particles here were primarily aggregates when observed by light microscopy. The **lyophilized** batch is in the smaller, 20 .mu.m range, which was shown superior surface activity in surfactant function tests compared to the non-**lyophilized** material of the same batch.

DETDESC:

DETD(116)

US PAT NO: 5,658,898 [IMAGE AVAILABLE] L3: 35 of 62

SUMMARY:

BSUM(74)

As . . . steps described above may be carried out and the purified
nanoemulsion may be converted into a dry preparation, especially a **lyophilisate**, which is reconstituted before administration by the addition of water. After reconstitution of the **lyophilisate** an administrable nanoemulsion is again obtained. For the preparation of

5,709,879 [IMAGE AVAILABLE] L3: 29 of 62

DETDESC:

DETD(146)

In an this experiment, a composition consisting of an oil-in-water emulsion containing MTP-PE, designated MF79/1501 (10% squalene (v/v); 1% **Tween** .RTM. 80 (v/v); 5% Tetronic.RTM. 1501 (a polyoxyetheylene-polyoxypropylene block polymer) (v/v); 400 .mu.g/ml MTP-PE), combined with the gD2 antigen was tested for immunogenicity. This composition was then compared to the combination of MF79/1501 plus fusogenic **liposomes**. This **liposome**-gD2 mixture was used to immunize animals three times at three-week intervals. As seen in Table 3, this combined emulsion-**liposome** immunization gave high antibody responses. After two immunizations the **liposome** plus emulsion group showed a mean titer of 7258, approximately three-fold higher than the M79/1501 emulsion alone. This response was. . . only 10%. After the third immunization, all animals in the group showed moderate increases in titer except one. Thus combining **liposomes** with emulsions offered significant increases in immune response.

DETDESC:

DETD(147)

. . .

2,338 + 810

4,747 + 1,823

5033	MF79/1501	(<5)	598	7,381	10,633
6155	+	(<5)	869	5,878	8,683
6510	**Liposomes**	(<5)	846	10,517	14,995
6519		(<5)	279	6,354	13,079
6628		(<5)	235	6,945	1,845
		(<5)	492 + 136		

. . . standard error.

.sup.b Bleed identification: Number of immunizations/weeks post immunization.

*2x MF59 = 10% squalene (v/v); 0.4% **Tween** 80 (v/v); 1.6% Span 85 (v/v); 400 .mu.g/ml MTPPE

DETDESC:

DETD(156)
1, MF79/121 (10% squalene, 1% **Tween** 80, 5% Pluronic L121, 400 .mu.g/ml MTP-PE); 2, MF79/121 combined with LPC (PC/PS (7:3) dialysis **liposomes**), such that 50% of the gD2 was associated with the **liposomes** and 50% of the gD2 was free in the aqueous phase of the formulation; 3, MF79/L121 combined with the LPC such that 100% of the gD2 was associated with the **liposomes**. As shown in FIG. 1, an increase in antibody titers is correlated with increased antigen association with **liposomes**. Geometric mean antibody titers for groups of 5 animals are given in Table 5. Enhanced Ab titers persisted for at. . .

DETDESC:

DETD(165)

In . . . after the third immunization. Animals were bled ever; 2 weeks throughout the experiment. One group received MF79/1501 (10% squalene, 1% **Tween** 80, 5% Tetronic 1501, 400 .mu.g/ml MTP-PE) combined with LPE FTF **liposomes** containing gp120/SF2. The other two groups received either 2XMF39 (10% squalene, 1% **Tween** 80, 1% Span 83, 400 .mu.g/ml MTP-PE) or SAF emulsion (10% squalene, 5% Pluronic L121, 0.4% **Tween** 80, 500 .mu.g/ml MDP). FIG. 3 illustrates that MF79/1501 and **liposomes** produced consistently higher titers than the other 2 emulsion formulations. Table 7 lists both individual animal titers and mean titers. . .

DETDESC:

DETD(169)

Rabbits . . . of groups 2 and 3, Table 8, shows that again immunogenicity was enhanced by combining emulsion MF59 (5% squalene, 0.5% **Tween** 80, 0.5% Span 85, 400 .mu.g/ml MTP-PE) with gD2 prepared with LPE. Including MTP-PE in the emulsion, group 3, or the **liposomes**, group 4, did not produce significant changes in immunogenicity. Group 6 was immunized once with LPE and then boosted 2. . . animals first receiving an injection of MF59 followed by 2 immunizations with LPE. Clearly, priming with emulsion and boosting with **liposomes** is more immunogenic than the converse. The earlier cited Goat gB2 experiment (example 13, Table 6, group 8) confirms poor response when the animals were primed with **liposomes** and boosted with emulsion.

DETDESC:

DETD(183)

To assess the physical stability of a mixture of emulsion particles and **liposomes**, we prepared PE/GS (8:2) /MTP-PE (PL/MTP-PE, 20:1) dialysis